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Abstract: The purpose of this study was to examine the occurrence of sheep persistently infected with Border disease virus (BDV) on 76 mixed cattle and sheep farms and whether seroconversion to BDV infection occurred in cattle of these farms. Seroprevalence of BDV and bovine viral disease virus (BVDV) infection in sheep was also investigated. Quantitative RT-PCR for pestivirus detection and an ELISA to detect pestivirus antibodies were used in 2'384 and 2'291 ovine blood samples, respectively. Another 27 seropositive sheep from ten flocks underwent serum neutralization testing to differentiate between BDV and BVDV antibodies. A BDV titre that was at least four times higher than the BVDV titre was interpreted as the result of BDV infection. Titres against BVDV were interpreted in an analogous fashion. All examined sheep were pestivirus-negative, 310 sheep were seropositive, 119 had an indeterminate titre and 1'862 were seronegative. The flock seroprevalence ranged from 0.0 to 73.9 %. Three of the 27 flocks that underwent serum neutralization testing were interpreted as BDV-infected because of 6 sheep with higher BDV titres, and 6 flocks were interpreted as BVDV-infected because of 14 sheep with higher BVDV titres.

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Investigation of border disease and bovine virus diarrhoea in sheep from 76 mixed cattle and sheep farms in eastern Switzerland

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Summary

The purpose of this study was to examine the occurrence of sheep persistently infected with Border disease virus (BDV) on 76 mixed cattle and sheep farms and whether seroconversion to BDV infection occurred in cattle of these farms. Seroprevalence of BDV and bovine viral disease virus (BVDV) infection in sheep was also investigated. Quantitative RT-PCR for pestivirus detection and an ELISA to detect pestivirus antibodies were used in 2'384 and 2'291 ovine blood samples, respectively. Another 27 seropositive sheep from ten flocks underwent serum neutralization testing to differentiate between BDV and BVDV antibodies. A BDV titre that was at least four times higher than the BVDV titre was interpreted as the result of BDV infection. Titres against BVDV were interpreted in an analogous fashion. All examined sheep were pestivirus-negative, 310 sheep were seropositive, 119 had an indeterminate titre and 1'862 were seronegative. The flock seroprevalence ranged from 0.0 to 73.9 %. Three of the 27 flocks that underwent serum neutralization testing were interpreted as BDV-infected because of 6 sheep with higher BDV titres, and 6 flocks were interpreted as BVDV-infected because of 14 sheep with higher BVDV titres.

Keywords: Border disease, bovine virus diarrhoea, sheep, cattle, virus detection, serology

Untersuchung auf Border Disease und BovineVirusdiarrhoe bei Schafen in 76 Betrieben der Ostschweiz

Das Ziel der vorliegenden Untersuchung war es, in Betrieben mit gleichzeitiger Schaf- und Rinderhaltung abzuklären, ob persistent mit Border Disease (BD) infizierte Schafe vorkommen, und, falls ja, ob die Rinder in diesen Betrieben Antikörper gegen BDV aufweisen. Im Weiteren interessierte die Seroprävalenz der Schafe in Bezug auf BDV- und BVDV-Antikörper. Die Untersuchungen wurden in 76 Betrieben mit gleichzeitiger Schaf- und Rinderhaltung durchgeführt.

2'384 Blutproben von Schafen wurden mittels quantitativer RT-PCR auf Pestivirus und 2'291 Proben mittels ELISA auf Antikörper gegen Pestivirus untersucht. Weitere 27, im ELISA positive Blutproben aus 10 Betrieben wurden mittels SNT untersucht, um abzuklären, ob die Antikörper gegen BDV oder BVDV gerichtet waren. Bei Schafen, deren Titer gegen BD-Virus mindestens vier Mal so hoch waren wie gegen BVD-Virus, wurde eine durchgemachte Infektion mit BD-Virus angenommen. Die Beurteilung der BVDV-Titer erfolgte in analoger Weise. Alle auf Pestivirus untersuchten Schafe waren Virus-negativ. Bei der Untersuchung der Proben im ELISA waren 310 Proben serologisch positiv, 119 verdächtig und 1'862 negativ. Die Seroprävalenz der Betriebe schwankte zwischen 0.0 und 73.9 %. Bei der Untersuchung von 27 seropositiven Proben im SNT wiesen 6 Proben aus 3 Betrieben einen mehr als vierfach höheren Antikörpertiter gegen BDV als gegen BVDV auf. In 14 Proben aus 6 Betrieben war der Titer gegen BVDV mehr als viermal so hoch als gegen BDV. Aufgrund dieser Befunde muss in 3 Betrieben von einer BDV- und in 6 Betrieben von einer BVDV-Infektion der Schafe ausgegangen werden.

Schlüsselwörter: Border Disease, Bovine Virusdiarrhoe, Schaf, Rind, Virusnachweis, Serologie

Introduction

The bovine virus diarrhoea virus (BVDV) and the Border disease virus (BDV) of sheep are pestiviruses that cross the species barrier and thus can cause cross-infection between cattle and sheep (Carlsson, 1991; Carlsson and Belák, 1994; Campell et al., 1995; Paton et al., 1997). Transmission of BVDV from cattle to sheep under natural conditions has long been recognized (Løken, 1995), and recent investigations have indicated that BDV can be transmitted to cattle on farms where the two species are kept together (Krametter-Frötscher et al., 2008; Reichle, 2009). Communal alpine pasturing of cattle and sheep persistently infected with BDV has been shown to result in seroconversion in the former (Büchi, 2009; Braun et al., 2012). As a result of the national control program initiated in 2008, it is expected that BVDV will soon be eradicated in Switzerland. There will likely be an increase in the importance of sheep as a source of pestivirus infection in cattle, especially when the two species are pastured together or kept on the same farm. The latter circumstances are believed to be risk factors for the transmission of BDV from sheep to cattle. The goals of this study were therefore to examine the prevalence of sheep persistently infected with BDV on farms with cattle, and to investigate whether persistently-infected sheep cause seroconversion in cattle. The seroprevalence of BDV and BVDV infection in sheep was also examined.

Animals, Material and Methods

Farms and animals

Seventy-six mixed sheep and cattle farms in eastern Switzerland were investigated between February 1, 2010 and January 31, 2011 (Schenk, 2012). There were 2'608 sheep, primarily of the Weisses Alpenschaf and Braunköpfiges Fleischschaf breeds, and the median flock size was 34.3 (range, 4 to 350) sheep. There were 2'585 cattle, mostly Swiss Braunvieh, and the median herd size was 34 (range, 2 to 130) cattle. Before the start of the study, all cattle had tested negative for pestivirus antigen by RT-PCR or an antigen ELISA as part of the national control program. Sheep and cattle were kept in the same barn on 12 farms (Fig. 1), in separate barns located in the same building on 20 farms and in separate buildings on 44 farms. The sheep and cattle of 7 and 42 farms, respectively, were kept on alpine pastures during the summer months, and at least one calf with persistent BVDV infection had been diagnosed in the previous years on 28 farms.

Blood testing

In all sheep, 9 ml blood was collected from a jugular vein into an evacuated EDTA tube and tested for pestivirus antigen and antibody. Additionally, 27 seropositive sheep from ten flocks with a high seroprevalence underwent a serum neutralization test (SNT) to differentiate between BDV and BVDV antibodies. The authors planned to test cattle from farms with BD virus-positive sheep for pestivirus antibody using an ELISA and to test seropositive cattle using a SNT to identify the antibody. However, all sheep were BD virus-negative (see Results) and testing of cattle was omitted.

Testing for viral DNA in blood of sheep

A total of 2'384 ovine blood samples underwent quantitative RT-PCR at the Institute of Veterinary Virology, University of Berne, to test for pestivirus as recently described (Büchi, 2009).

ELISA and serum neutralisation test

An ELISA was used to test 2'291 ovine blood samples for pestivirus antibody. Twenty-seven ELISA-positive blood samples from 10 flocks underwent a SNT to differentiate between BDV and BVDV antibodies. Testing was done in the laboratory identified above (Büchi, 2009). Because of cross-neutralization between BVDV and BDV attributable to genetic similarities between the viruses, only sheep with a BDV titre that was at least four times higher than the titre against BVDV were considered infected with BDV. A BDV titre that was two to four times higher than the BVDV titre was interpreted as a likely BDV infection. The interpretation of BVDV titres was done in an analogous fashion.

Statistical analysis

The program StatView 5.1 (SAS Institute, Wangen, Switzerland) was used for statistical evaluation. The means, standard deviations and frequency distributions were calculated for the variables studied and differences were analysed using analysis of variance (ANOVA) and the Bonferroni-Dunn post hoc test. The Wilk Shapiro test was used to test distributions for normality. Results of normally distributed variables are given as mean ± standard deviation and results of variables with a skewed distribution as median and range. The level of significance was set at $P < 0.05$.

Results

Virus prevalence and seroprevalence of pestivirus infection in sheep

All 2384 sheep tested negative for pestivirus.

Of the 2'291 sheep tested for pestivirus antibody (ELISA), 310 (13.5 %) were seropositive, 119 (5.2 %) had an indeterminate result and 1'862 (81.3 %) were negative. The flock seroprevalence ranged from 0.0 to 68.8 % (Fig. 2). Twenty-three flocks had a seroprevalence of 0 %.

Serum neutralization test

Of the 27 seropositive sheep tested by serum neutralization, 6 (from flocks 8, 36 and 47) had a BDV titre that was more than four times higher than the BVDV titre (Tab. 1), and 14 (from flocks 6, 22, 29, 30, 51 and 67) had a BVDV titre that was more than four times higher than the BDV titre. This was interpreted as the result of BDV and BVDV infection of these 3 and 6 flocks, respectively. The interpretation of the SNT was not possible in one flock (No. 27) because both serum neutralization virus titres were high.

Effect of proximity of stabled cattle and sheep on seroprevalence

Sheep housed in barns with cattle had a higher seroprevalence of pestivirus infection than sheep kept separate from cattle (27.4 versus 14.3 %; $P < 0.05$). Significantly fewer sheep kept in separate barns had positive SNT titres against BVDV than sheep housed in barns with cattle ($P < 0.05$). Barn management did not affect seroprevalence of BDV infection in sheep.

Discussion

The rationale of this study was based on the previous observation that sheep grazing together with cattle on alpine communal pastures can infect cattle with BDV (Büchi, 2009; Braun et al., 2012). The main goals were to investigate the prevalence of sheep persistently infected with BDV on mixed cattle and sheep farms in eastern Switzerland and to determine the potential of seroconversion and the

137 birth of persistently infected offspring in cattle. To our surprise, there were no BDV-infected sheep
138 despite a previous report of the endemic occurrence of this virus in Swiss sheep flocks (Schaller et al.,
139 2000) and a 0.68 % BDV prevalence in sheep from 4 communal alpine pastures in central
140 Switzerland (Büchi, 2009; Braun et al., 2012). Similar BDV prevalences were determined in Austria
141 (0.32 %; Krametter-Frötscher et al., 2007), Spain (0.3 to 0.6 %; Valdazo-Gonzáles et al., 2006) and
142 Turkey (up to 2 %; Oguzoglu et al., 2009). However, the detection of specific BDV antibodies in 3
143 flocks is a strong indication that BDV infection had occurred in the past or that the sheep had been
144 exposed to the virus during transport with other sheep, communal pasturing or at shows. Because
145 there were no BDV-infected sheep, the planned testing of cattle was no longer justified and therefore
146 omitted. Nevertheless, there were no persistently infected calves born during the study period in any
147 of the herds indicating that cattle were not exposed to pestivirus or that infections did not become
148 established.
149 The seroprevalence of pestivirus antibody in sheep was 18.7 %, which was comparable to results of
150 previous studies from Switzerland (16 to 20 %; Schaller et al., 2000; Danuser et al., 2009) and Spain
151 (Valdazo-Gonzáles et al., 2008). Regional differences have been described in Austria, where the
152 seroprevalence ranged from 16.3 % in Carinthia (Schleiner et al., 2006) to 67.6 % and 83.0 % in
153 Vorarlberg before and after alpine communal pasturing, respectively (Krametter-Frötscher et al.,
154 2007). We cannot explain why we were unable to identify the BDV carriers and shedders among the
155 tested sheep to account for the observed seroprevalence of pestivirus antibody. It is possible that there
156 were virus carriers that died at an early age and thus escaped testing, or that infection occurred during
157 communal alpine pasturing or at shows.
158 The results of the SNT were crucial for this study because they allowed differentiation of the
159 pestivirus antibodies. In agreement with a report from Austria (Schleiner et al., 2006), in which 61.8
160 % of 249 seropositive sheep had a higher titre against BVDV than against BDV, and 22.1 % had a
161 higher titre against BDV we recorded twice as many BVDV seropositive flocks than BDV
162 seropositive flocks. In contrast to a recent investigation in Switzerland using 5'059 sheep (Danuser et
163 al., 2009), only 12.9 % of the seropositive sheep had a higher titre against BVDV than against BDV,
164 and 56.1 % had a higher titre against BDV (Danuser et al., 2009). In sheep that were housed in barns
165 with cattle, the seroprevalence of pestivirus infection was almost twice as high as in sheep that were
166 kept separate from cattle (27.4 versus 14.3 %), and significantly fewer sheep kept in separate barns
167 had positive SNT titres against BVDV than sheep housed with cattle. However, barn management
168 had no effect on seroprevalence of BDV infection. Taken together, these findings show that housing
169 sheep separate from cattle significantly reduces seroprevalence of BVDV infection, but not of BDV
170 infection in sheep.

Taken together, these findings show that housing sheep and cattle separately significantly reduces seroprevalence of BVDV infection, but not of BDV infection in sheep. Sheep that were housed together with cattle had a much higher prevalence of BVDV-specific antibodies than sheep that were housed separately from cattle, but there was no difference between the two groups of sheep with respect to BDV-specific antibodies. How the sheep became infected with BVDV is unknown. The study period from February 2010 to January 2011 was after the initiation of the national BVDV eradication program in 2008, and therefore BVDV-positive cattle should have been very rare in the cattle population. Furthermore, there were no persistently infected calves during the study period. Therefore, we assume that the sheep had been in contact with persistently infected cattle earlier in life.

The investigation of the seroprevalence of BDV infection in cattle under various management conditions requires further study. The lack of BDV-infected sheep in this study precluded the testing of our hypothesis that sheep can cause BDV infection in cattle when the two species are kept together on the same farm. Regardless of whether persistently-infected offspring are born, interspecies transmission of pestiviruses is possible and must be considered in the interpretation of serological results in the context of eradication programs.

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Legend to figures

Figure 1: Sheep and cattle kept in the same barn.

Figure 2: Frequency distribution of the seroprevalence of pestivirus infection based on ELISA testing of 2'291 sheep from 76 flocks.

Table 1: Comparison of SNT titres against BDV and BVDV in 27 sheep from 10 flocks with high seroprevalence of pestivirus infection.

Flock No.	Sheep No.	SNT titre			Interpretation: Infection of flock with
		BDV	BVDV	Quotient (higher/lower)	
6	14	144.0	601.0	4.2	BVDV
	15	362.0	645.0	1.8	
	17	< 16.0	512.0	> 32.0	
8	7	609.0	59.5	10.2	BDV
	15	197.0	< 16.0	> 12.3	
	18	2700.0	197.0	13.7	
	19	90.5	< 16.0	> 5.7	
22	6	64.0	1630.0	25.5	BVDV
	10	50.8	724.0	14.3	
	25	323.0	1450.0	4.5	
27	3	181.0	369.0	2.0	Interpretation
	17	203.0	161.0	1.3	not possible
29	11	102.0	1320.0	12.9	BVDV
	19	16.0	456.0	28.5	
	21	40.3	1020.0	25.3	
30	6	411.0	2048.0	5.0	BVDV
	11	71.8	323.0	4.5	
	12	323.0	813.0	2.5	
36	8	161.0	24.7	6.5	BDV
47	4	128.0	38.1	3.4	BDV
	10	178.0	< 16.0	> 11.1	
	11	1600.0	1150.0	1.4	
51	3	181.0	2300.0	12.7	BVDV
	6	1150.0	1200.0	1.0	
	9	90.5	2580.0	28.5	
67	1	20.0	218.0	10.9	BVDV
	2	< 16.0	323.0	> 20.2	